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STUDIES IN CEREAL DISEASES

II

Root-Rots and Foot-Rots of Wheat in Manitoba

by

F. J. GREANEY AND D. L. BAILEY

DOMINION RUST-RESEARCH LABORATORY

MANITOBA AGRICULTURAL COLLEGE

WINNIPEG, MANITOBA

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DIVISION OF BOTANY

DOMINION EXPERIMENTAL FARMS

DOMINION OF CANADA

DEPARTMENT OF AGRICULTURE

BULLETIN No. 85—NEW SERIES

Published by the Direction of the Hon. W. R. Motherwell, Minister of Agriculture,
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TABLE OF CONTENTS

	Page
Introduction	5
Previous work	5
Objects of the investigations	6
Experimental methods and materials	6
Experimental results	7
Fungi associated with wheat root-rots	7
Influence of crop rotations on the fungous flora of wheat roots	8
Identity of root-rotting organisms	9
Pathogenicity studies with organisms isolated from wheat roots	16
Their relative pathogenicity to wheat	16
Relative susceptibility of various cereals to the same organism	21
Control	26
Influence of soil sterilization on the virulence of attack	26
Relation of seed treatment to the severity of attack	27
Discussion and conclusions	28
Summary	30
Literature cited	32

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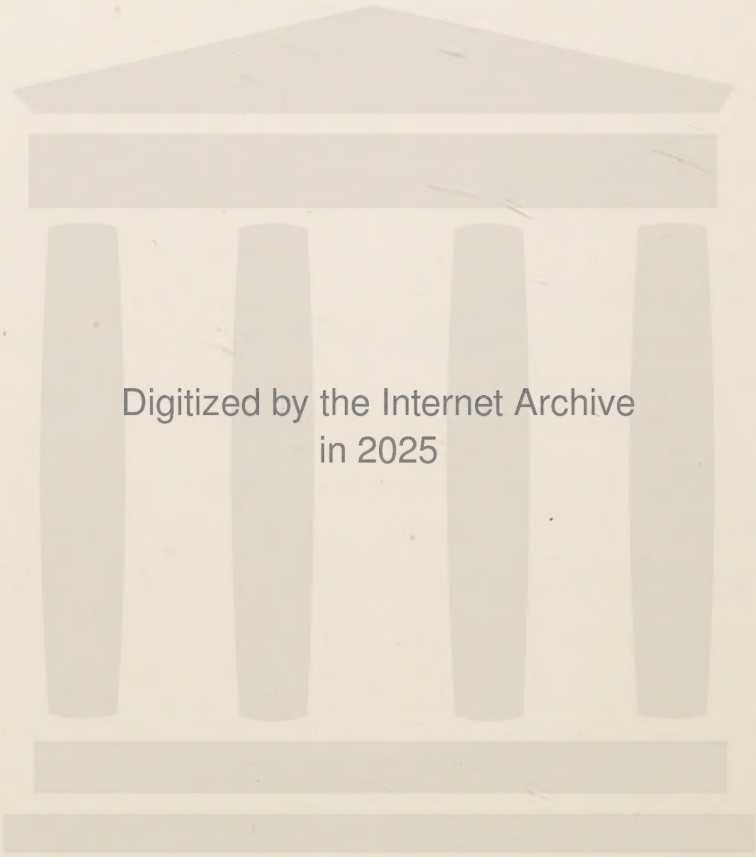
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Root-Rots and Foot-Rots of Wheat in Manitoba

INTRODUCTION

In Manitoba root-rots of wheat and other small grains have attracted little attention, and, as a consequence, little investigational work has been done on them. Plant-disease surveys[7] during the past few years, however, have indicated that root- and foot-rots have been responsible in many cases for considerable reduction in yields of wheat, oats, and barley. They tend also to become more destructive with the continued production of cereals in this province. The problem seems particularly acute with wheat. The present work was undertaken with the hope of delimiting in a preliminary way the wheat root-rot problem in Manitoba.

The general problem of root- and foot-rots of small grains has already received considerable attention in America and elsewhere. It has been carefully reviewed by Henry[19], whose work also contains a comprehensive bibliography on this subject. Consequently only some of the literature bearing on the root- and foot-rot problem in the wheat-growing belt of the United States and Canada will be mentioned.

PREVIOUS WORK

The first definite foot-rot disease of wheat was reported from Oregon in 1902, by Cordley[10]. He spoke of it as foot-rot and stated that a fungus was associated with the disease. He did not attempt to determine the causal organism. Some years later, in 1912 and 1913, Bolley[4, 5] drew attention to the seriousness of root- and foot-rots of wheat in North Dakota. He reported that species of *Helminthosporium* were very destructive pathogenes to wheat roots. He also found that *Fusarium spp.* were important root-rotting organisms on wheat and other cereals. He stated[5] that constant cropping of wheat on the same soil caused wheat sickness and resulted in wheat-sick soil. Bolley found that such a condition was caused by fungi, especially *Helminthosporium, Alternaria, Fusarium,* and *Colletotrichum.*

It was not until the discovery of wheat rosette by Humphrey and Johnson[20], in 1919, that plant pathologists directed their attention to a more thorough study of the foot- and root-rot diseases. According to the work of Dreschler[12] and McKinney[25], Johnson[22] was the first to show that *Helminthosporium sativum* is pathogenic on wheat plants. In 1920, Atanasoff[1] reported that the Fuarium stage of *Gibberella Saubinetii* causes a foot-rot of wheat. The same writer[2] found that many species of this genus are destructive pathogenes to the roots of wheat plants. Very interesting and important studies have been made at Wisconsin by Dickson[11], Johnson and Dickson[23] and other investigators, on the factors influencing the pathogenicity of *Gibberella Saubinetii,* the organism causing seedling blight of wheat and corn. The study of the factors predisposing plants to foot- and root-rot diseases is one of first importance in attempting to develop thorough means of control for these diseases.

The occurrence of a destructive foot- and root-rot of wheat was reported by Stevens[32] in 1920. The same year Louise J. Stakman[29] reported that in

Minnesota the roots of wheat were frequently infected with a species of *Helminthosporium* similar to or identical with *H. sativum* P.K. and B. She also found that *Fusarium culmorum* (W.G. Sm.) Sacc., and other fungi were very destructive to the roots of durum wheat seedlings in agar cultures[29]. Henry[19] isolated *Helminthosporium sativum* from wheat, rye, and barley in Western Canada. Simmonds[28] working in Saskatchewan was able to isolate from cereals showing severe root-rot an organism resembling very closely *Fusarium culmorum* W. Smith. He states that several species of *Fusarium* are associated with the foot- and root-rot so commonly found in the extensive wheat regions of Western Canada. Christensen[8] found the disease caused by *H. sativum* to be very widely distributed not only in America, but also in other wheat-growing countries. He found that wheat, barley, and rye were the most susceptible of the cereals to *H. sativum*, while oats was either immune or highly resistant. He found that both primary and secondary roots of wheat, barley, and rye were severely rotted. Dosdall[13] has studied intensively the factors influencing the pathogenicity of *H. sativum*, and gave particular attention to root- and foot-rots. In general, she found that the disease caused greatest injury when conditions for host development were unfavourable. Raeder[27] reported from Idaho a *Helminthosporium* on wheat, causing considerable injury to the growing plants. Haskell and Wood[18] found a *Helminthosporium* associated with root-rot in Utah. From the work of Bolley[4], Beckwith[3], Henry[19], and Stakman[30], it is very evident that several genera of fungi are associated with foot- and root-rots of wheat. The studies of these workers clearly indicate that many of these fungi are parasitic on wheat seedlings and cause economic losses of increasing importance. A recent survey of the problem by McKinney[26] shows that foot-rot diseases of wheat are rather widespread in America, causing losses of economic importance in several of the principal wheat-growing regions.

Many investigators have reported a discoloration and rotting of the roots of wheat plants as one of the symptoms of the true take-all disease of wheat[21, 24]. Stevens[31] cites numerous papers in which mention is made of the blackening and death of wheat roots attacked by *Ophiobolus*. The true take-all disease of wheat was first found in the United States in 1919. As yet, the disease has not been found in the hard spring-wheat belt of the United States. Fraser[14], in 1923, collected from one field of Marquis wheat in northern Saskatchewan specimens of diseased wheat roots. A close study of these revealed abundant mycelium and spore-cases of the fungus, *Ophiobolus cariceti* (Berk. and Br.) Sacc. In 1924, the same author[15] reported the presence of this disease in several localities in northern Saskatchewan. Preliminary surveys, undertaken in 1925 and 1926, indicate that the disease is quite widely spread in the spring-wheat regions of Manitoba. The problem as a whole has been found to be exceedingly complex, and the factors influencing the pathogenicity of the various fungi involved are still very imperfectly understood.

OBJECTS OF THE INVESTIGATIONS

The particular objects of the investigations were to determine: (1) the fungous flora of wheat roots in Manitoba, (2) the influence of various crop rotations on the fungous flora of wheat roots, and (3) the pathogenicity of the fungi isolated.

EXPERIMENTAL METHODS AND MATERIALS

Isolations were made by the tissue method. The bits of tissue consisted of a few centimeters of the base of the stem and some primary or secondary roots. These pieces were washed in water, rinsed in 50 per cent alcohol, and then immersed for two minutes in a 1:1000 solution of mercuric bichloride. The material was then washed twice with sterile water and planted in petri dishes

on two per cent potato dextrose agar. Five portions were placed in each petri dish. The dishes were incubated at temperatures ranging from 20°-22°C., and examined from five to seven days later. Wherever it was impossible to identify the fungi present at that time, transfers of the unidentified fungi were made to tubes of potato dextrose agar and these were examined subsequently.

In determining what fungi were commonly associated with wheat root-rots in Manitoba, pure culture isolations were made from a number of wheat roots collected at various places in the province. In many instances the isolations were made from severely infected material submitted by growers for diagnosis. In other cases the isolations were made from material collected especially for the purpose, and, in such cases, an attempt was made to make the material representative of centres of the less severe as well as of the more severe infections.

The material used in the study of the influence of crop rotations on the fungous flora of wheat roots was obtained from the cultural plots at the Manitoba Agricultural College. This material was made available through the courtesy of Prof. J. H. Ellis to whom grateful acknowledgment is made. Shortly after harvest a large random sample of roots was collected from the various plots. From this sample one hundred roots were selected at random, and isolations were made from these in the manner just described. Subsequently a second hundred roots were selected and cultured in the same way.

EXPERIMENTAL RESULTS

Fungi Associated with Wheat Root-Rots

The results of a number of isolations from diseased wheat roots from various localities are summarized in table 1. The localities are widely scattered and are representative of the most important wheat-growing areas in the province. The apparently universal association of *Fusarium* and *Helminthosporium* with wheat root-rots throughout this area is the most interesting observation.

Table 1.—THE PERCENTAGE OF WHEAT ROOTS INFECTED WITH VARIOUS FUNGI IN WESTERN CANADA

District	Percentage of roots infected and fungi isolated			
	Number of roots cultured	Total per cent infected	Fusarium sp.	Helminthosporium sp.
Morden, Man.	30	90·0	53·3	36·7
Napinka, Man.	25	40·0	20·0	20·0
Manitoba Agricultural College, Winnipeg	62	91·9	58·0	33·9
Portage Plains (a)	36	100·0	44·5	55·5
Portage Plains (b)	22	100·0	45·4	54·6
Oak Bluff, Man.	30	46·6	33·3	13·3
Sperling, Man.	10	80·0	70·0	10·0
Elva, Man.	10	30·0	10·0	20·0
Adelaide, Sask.	15	33·3	20·0	13·3
Southern Saskatchewan and Manitoba	75	45·3	34·7	10·6

However, during the season of 1925, true take-all *Ophiobolus cariceti* (Berkely and Broome) Saccardo, was collected at the following points in Manitoba: Laurier, Ashville, Kelwood, Riding Mountain Station, Neepawa, Snyders Crossing, McCreary, and Dauphin[17]. In some cases the injury was very severe, the loss occasioned being at least 60 per cent; in other cases a mere trace or a light scattered infection was present. From this preliminary survey it is evident that take-all may prove a significant factor in the northerly park country. However, *Fusarium* and *Helminthosporium* seem much more widely distributed and destructive, and hence are of primary importance.

The Influence of Crop Rotations on the Fungous Flora of Wheat Roots

In 1923, samples of wheat roots were collected from each of the following plots in a rotation series: first, second, third, and fifth crops of wheat following summer-fallow; and also from crops of wheat following oats, barley, rye, flax, corn, potatoes, turnips, sunflowers, grasses, and clovers. From each of these samples the fungous flora of one hundred roots was determined. A similar determination of one hundred roots was made at a later period. In 1924, samples of wheat roots were collected, and the fungous flora determinations were made by following the same procedure. The data obtained in this experiment are summarized in tables 2 and 3.

Table 2.—THE RELATION BETWEEN VARIOUS CULTURAL ROTATIONS AND THE PERCENTAGE OF WHEAT ROOTS INFECTED BY ROOT-ROTTING FUNGI

Previous crop history of field	Percentage of infected roots*					
	1923			1924		
	Series		Average	Series		Average
	1	2		1	2	
First crop after fallow	70·0	71·0	70·5	39·0	52·0	45·5
Second "	76·0	77·0	76·5	74·0	76·0	75·0
Third "	87·0	77·0	82·0	76·0	77·0	76·5
Fifth "	92·0	85·0	88·5	.....	.....	.....
Sixth "	.....	.....	.....	83·0	85·0	84·0
Third crop after sod	60·0	.....	60·0	.....	.....	.....
First " oats	89·0	82·0	85·5	86·0	84·0	85·0
" " barley	94·0	83·0	88·5	64·0	63·0	63·5
" " rye	75·0	71·0	73·0	80·0	75·0	77·5
" " flax	88·0	79·0	83·5	91·0	89·0	90·0
" " corn	65·0	64·0	64·5	78·0	74·0	76·0
" " potatoes	60·0	64·0	62·0	78·0	72·0	75·0
" " turnips	63·0	59·0	61·0	70·0	66·0	68·0
" " sunflowers	69·0	71·0	70·0	64·0	74·0	69·0
" " grasses	71·0	70·0	70·5	74·0	73·0	73·5
" " clovers	69·0	69·0	69·0	71·0	73·0	72·0

*Isolations were made from 100 roots in each series.

Table 3—THE INFLUENCE OF CULTURE ROTATIONS ON THE FUNGOUS FLORA OF WHEAT ROOTS

Source of wheat roots	Percentage of roots infected*					
	Helminthosporium		Fusarium		Miscellaneous fungi	
	1923	1924	1923	1924	1923	1924
First crop after fallow	12·0	27·0	51·0	22·0	5·0	3·0
Second "	25·0	45·0	40·0	43·0	5·0	1·0
Third "	15·0	32·0	54·0	49·0	6·0	4·0
Fifth "	13·0	.....	72·0	.....	4·0	.....
Sixth "	.....	36·0	.....	68·0	.....	2·0
Third crop after sod	9·0	.....	49·0	.....	6·0	.....
First " oats	19·0	31·0	81·0	69·0	6·0	2·0
" " barley	31·0	45·0	64·0	45·0	1·0	.....
" " rye	28·0	20·0	57·0	71·0	.....	3·0
" " flax	32·0	61·0	70·0	86·0	1·0	3·0
" " corn	25·0	22·0	51·0	72·0	.....	.....
" " potatoes	15·0	33·0	45·0	65·0	.....	1·0
" " turnips	17·0	33·0	63·0	76·0	.....	.....
" " sunflowers	15·0	2·0	59·0	68·0	.....	1·0
" " grasses	19·0	5·0	58·0	65·0	1·0	1·0
" " clover	7·0	8·0	63·0	78·0	.....	2·0

*Isolations were made from 100 roots each year.

In table 2, a comparison is made of the percentage of infected roots which were found to be present in the samples from various plots. Table 3 summarizes the relative frequency of occurrence of the most commonly occurring pathogenes isolated from each of the above samples. From table 2 it will be noticed that in 1923, while there seemed to be some correlation between the continuous cultivation of wheat on the same soil and an increase in root rots, the correlation was not very marked and did not seem very significant.

In 1924, however, fallowing seemed to have exerted a marked influence in reducing root-rot infections in the first crop following the fallow. The percentages of infection in subsequent crops of wheat were were very comparable with those of 1923. In 1923 root-rots seemed less prevalent when wheat followed an intertilled crop, but no such correlation was evident from the 1924 results.

Considering the two years' results, there does not seem to be any conspicuous correlation between the severity of root-rot infection and the cultural practice involved; nor is there any convincing indication that there is any marked tendency for root-rotting organisms to accumulate in the soil during six years' continuous cultivation of wheat.

From a study of table 3 it is evident that in the great majority of cases either *Fusarium* or *Helminthosporium* was associated with the root-rots. These fungi were consistently present, *Fusarium* tending to occur even more frequently than *Helminthosporium*. Neither of these groups of fungi seem to have been significantly influenced by any of the systems of cultivation studies.

The Identity of Root-Rotting Organisms

The problem of identifying the various fungi found to be associated with wheat root-rots was considerably simplified by the relatively small number of different ones which occurred. In general, only the genus was determined, and no attempt was made to determine species. More attention was paid to the identification of the cultures of *Fusarium* and *Helminthosporium* than to the identification of the other cultures, since these organisms were more commonly associated with severe cases of root-rot.

There appears to be at least three distinct types of *Fusarium* among those isolated. The problem of identifying the *Fusaria* seemed fraught with too many difficulties to receive serious attention in the preliminary phases of these investigations.

Isolations of *Helminthosporium* were all of the *sativum* type.[33] Within this type, however, there were obvious and interesting variations in pathogenicity and cultural characteristics. Four cultures were chosen for a comparative study of morphological and cultural characteristics. Two of these cultures were found, in the pathogenicity studies described subsequently, to be virulent pathogenes, while the other two were much less virulent.

Eight uniform petri plates were poured with 20 cc. each of potato dextrose agar. Eight plates of bean agar and eight of prune agar were similarly prepared. Two plates of each type of medium were inoculated with the four cultures of *Helminthosporium* mentioned above. All of the plates were incubated at 22° C., and a comparison of the growth rate and gross cultural characters of the various cultures was made over a five-day period. A summary of the growth rate and cultural characters of these four cultures of *Helminthosporium sativum* is given in table 4, and a photograph of them after five days is shown in plate 1.

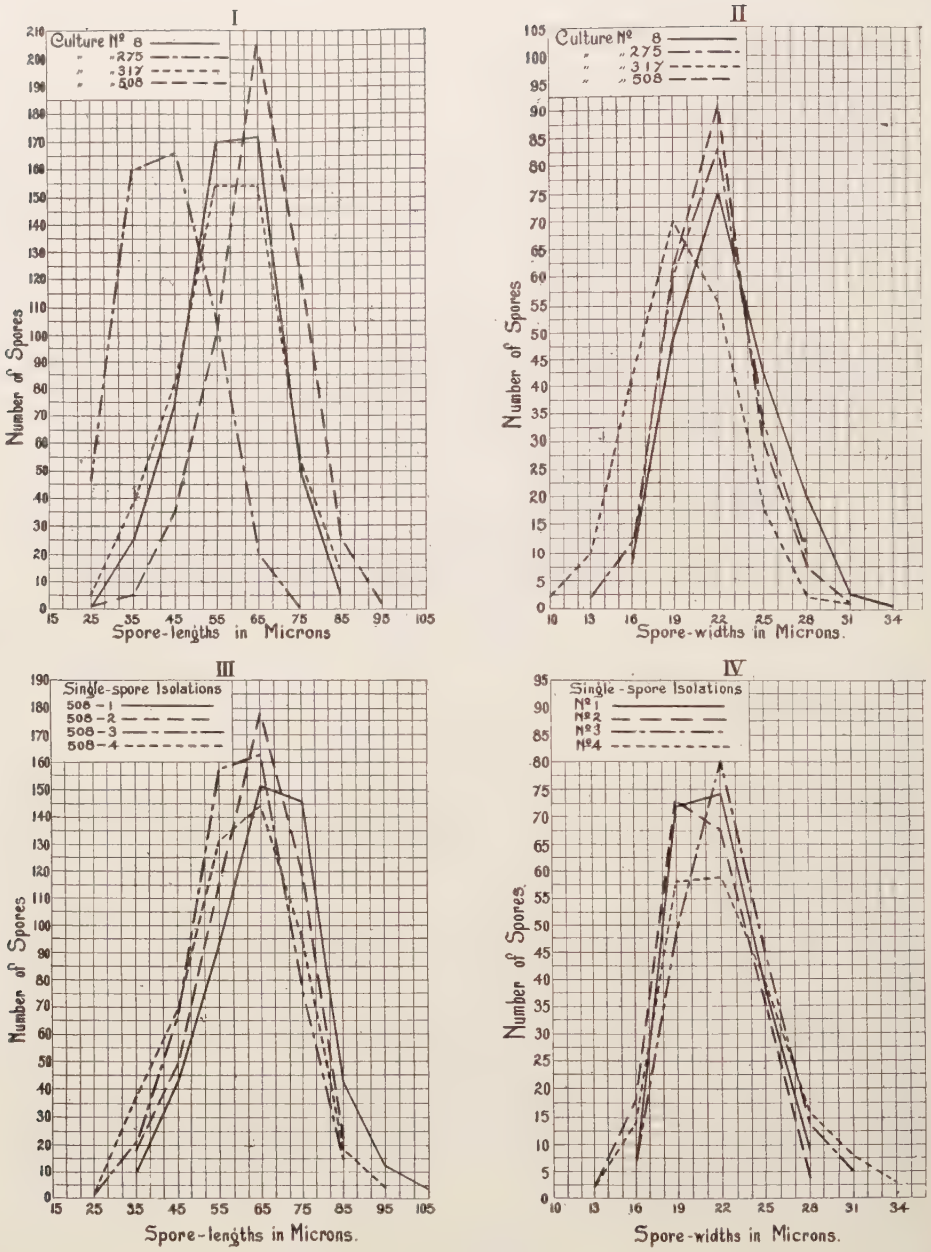
Comparative cultural characteristics of four cultures of *Helminthosporium* on three different media: Potato dextrose agar (left), bean agar (centre), prune agar (right). Top to bottom: Cultures 317, 275, 508, 8.

TABLE 4—CULTURAL CHARACTERISTICS OF FOUR CULTURES OF *HELMINTHOSPORIUM SATIVUM*

Culture number	Culture medium	Average diameter in cms. of seven-day-old colonies	Growth	Colour	Zonation	Margin
8	Potato dextrose agar	6·6	Vigorous, regular, uniform	Dark green to greyish green	Centre dark; wide zone of fluffy mycelium; outer zone more compact	Regular, narrow, white
508	" ..	4·5	Fairly vigourous; fluffy mycelium	Light grey.....	Not as sharp as number 8	Irregular, darker marginal zone with light border
275	" ..	5·0	Fairly vigorous.	Greyish white to greyish black	Central zone of cottony mycelium, not sharp	Lobed, irregular
317	" ..	6·2	Vigorous; fluffy aerial mycelium	Greyish black to greenish grey	Sharp, regular..	Regular, compact fine white narrow zone
3	Bean agar.....	6·2	Vigorous; uniformly fluffy mycelium	Olive green.....	Sharp, zones wide	Wide, regular, white
508	"	5·4	Fairly vigorous.	Dark olive green	No zonation.....	White, indistinct
275	"	4·3	Fairly vigorous; centre very compact	Greyish green...	No zonation.....	Regular, white
317	"	6·4	Vigorous, uniform	Greyish green centre to white margin	Sharp.....	Narrow white band
8	Prune agar.....	3·0	Scant.....	Greenish black.	Sharp, regular bands	Light in colour regular
508	"	2·7	Scant, compact.	Light grey centre to dark grey	Not sharp.....	Narrow white
275	"	2·2	Scant.....	Greyish centre surrounded by black band	Not sharp.....	Band greyish green colour
317	"	3·3	Scant.....	Greyish green...	Sharp, regular, distinct	Lighter in colour, regular

Judging from the cultural characteristics evidenced by these four cultures on the three media, each culture was distinctly different from the other three. Cultures 8 and 317, however, were quite similar except on bean agar, where a noticeable difference occurred. Therefore the indication was that these four cultures represented at least three, and probably four, physiologic forms of *Helminthosporium sativum*.

It seemed desirable to determine also whether these apparently significant cultural differences were accompanied by differences in the size of the conidia which were produced by the various cultures. Accordingly, a monosporous culture was developed from each of the four cultures. The length and width of 200 conidia from each culture were measured. The length of an additional 300 spores was measured because of the greater variability of this measurement. The measurements were made by the projection method in a dark room, the screen being placed so that the image was magnified 1,000 times. The measurement was made by calipers and the dimension read from a millimeter scale in microns. The results were examined statistically. Table 5 summarizes the variation and constants for the length of spores in the four cultures. Table 6 presents corresponding data on the spore width. A comparison of the various constants of length and width of spores of the four cultures is given in table 7. The spore lengths and widths represented in tables 5 and 6 are plotted in plate 2, I and II.



Relative conidial length and width of cultures of *Helminthosporium sativum.*

I. Relative conidial length of four cultures of *H. sativum.*
II. Relative conidial width of four cultures of *H. sativum.*
III. Relative conidial length of four monosporous cultures from one culture of *H. sativum.*
IV. Relative conidial width of four monosporous cultures from one culture of *H. sativum.*

TABLE 5.—FREQUENCY DISTRIBUTIONS AND CONSTANTS FOR LENGTH OF SPORES OF FOUR MONOSPOROUS CULTURES OF *HELMINTHOSPORIUM SATIVUM*

Culture number	Spore length class values (in microns)								Range	Constants			
	25	35	45	55	65	75	85	95		Mode	Mean	Standard deviation	Coefficient variability
8	1	25	75	170	172	51	6	....	27·0—88·6	65	58·28± 0·32	10·58± 0·23	17·06± 0·50
275	47	159	166	106	21	1	....	....	20·2—76·4	45	42·96± 0·31	10·25± 0·22	23·86± 0·54
317	5	37	81	154	154	54	15	....	24·5—89·5	55—65	57·74± 0·37	12·18± 0·26	21·09± 0·47
508	1	5	36	100	205	125	26	2	27·6—96·6	65	64·84± 0·32	10·51± 0·22	16·20± 0·35

TABLE 6.—FREQUENCY DISTRIBUTIONS AND CONSTANTS FOR WIDTH OF SPORES OF FOUR MONOSPOROUS CULTURES OF *HELMINTHOSPORIUM SATIVUM*

Culture	Spore-width class values (in microns)																									Range	Constants			
	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33			Mode	Mean	Standard deviation	Coefficient variability
8	..	..	..	..	..	..	3	6	12	18	19	22	27	26	19	15	9	12	7	1	3	..	..	1	16·0—33·6	22	22·39± 0·15	3·14± 0·10	14·02± 0·48
275	..	..	2	0	0	1	3	8	11	20	29	28	24	31	15	13	5	4	6	..	..	..	..	..	12·0—28·4	23	21·50± 0·13	2·83± 0·10	13·20± 0·45
317	1	1	3	0	7	11	7	23	24	21	25	27	15	14	13	4	1	2	0	0	1	..	..	..	10·6—30·5	21	19·47± 0·15	3·19± 0·11	16·38± 0·57
508	..	..	..	..	..	2	1	5	17	13	31	32	29	30	10	8	13	2	4	2	1	..	..	..	15·0—30·5	21	21·68± 0·13	2·73± 0·09	12·03 0·41

TABLE 7.—SUMMARY OF COMPARISONS BETWEEN THE MEAN LENGTHS AND WIDTHS OF THE SPORES OF FOUR CULTURES OF *HELMINTHOSPORIUM SATIVUM*

Cultures compared	Mean lengths		Mean widths	
	Difference in microns	Difference divided by the probable error of the difference	Difference in microns	Difference divided by the probable error of the difference
8 and 275	0·69±0·20	4·4	15·32±0·44	34·5
8 and 317	2·92±0·21	13·6	0·54±0·49	1·1
8 and 508	0·28±0·20	1·4	6·56±0·45	14·6
275 and 317	2·03±0·20	10·0	14·78±0·48	30·8
275 and 508	1·17±0·19	6·3	21·88±0·44	49·4
317 and 508	3·21±0·20	16·1	7·10±0·48	14·6

An examination of table 7 indicates that each culture was distinct morphologically from the other three. Cultures 8 and 317, which were somewhat similar in cultural characteristics, were also alike in spore width but were very different in spore length.. With the hope of learning something more of the significance of these morphological differences, further studies were made, beginning with the monosporous culture 508 which was used in the above experiments. From this culture four monosporous cultures were developed. The cultural characteristics of these four sub-cultures were studied on potato dextrose, bean and prune agars in exactly the same manner as that already described. Judging from their cultural characteristics, there seemed to be two different forms. Sub-culture 508-4 differed slightly from the other three. The morphological characteristics of these four cultures were compared and the results are presented in tables 8, 9, and 10. Sub-cultures 508-1, 508-2, and 508-3 differed from each other morphologically, while 508-4 appeared to be identical with 508-3. The spore lengths and widths represented in tables 8 and 9 are plotted in plate 2, III and IV.

TABLE 8.—FREQUENCY DISTRIBUTIONS AND CONSTANTS FOR LENGTH OF SPORES OF FOUR-MONOSPOROUS CULTURES OF *HELMINTHOSPORIUM SATIVUM* DERIVED FROM A SINGLE MONOSPOROUS CULTURE

Culture number	Spore-length class values (in microns)									Range	Constants			
	25	35	45	55	65	75	85	95	105		Mode	Mean	Standard deviation	Coefficient variability
508–1	....	10	43	92	151	146	43	12	3	32·8—101·0	65	66·44±0·39	12·79±0·27	19·25±0·42
508–2	....	18	49	115	178	118	22	.....	.....	30·0 — 86·5	65	62·90±0·35	11·46±0·24	18·22±0·40
508–3	1	21	66	157	162	78	15	.....	.....	25·0— 84·8	65	60·04±0·34	11·32±0·24	18·85±0·42
508–4	2	37	68	131	144	95	19	4	....	25·0— 93·0	65	60·18±0·39	13·11±0·28	21·78±0·49

TABLE 9.—FREQUENCY DISTRIBUTIONS AND CONSTANTS FOR WIDTH OF SPORES OF FOUR MONOSPOROUS CULTURES OF *HELMINTHOSPORIUM SATIVUM* DERIVED FROM A SINGLE MONOSPOROUS CULTURE

Culture number	Spore-length class values (in microns)																					Range	Constants			
	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34		Mode	Mean	Standard deviation	Coefficient variability
508–1	..	..	..	1	1	5	16	26	30	30	27	17	13	11	14	8	1	..	..	..	..	..	..	15·0— 28·9	20—21	21·54± 0·13	2·68± 0·09	12·44± 0·43
508–2	..	..	2	2	5	11	17	19	37	23	26	19	16	11	8	4	..	..	..	..	..	..	..	14·0— 27·0	20	20·99± 0·13	2·74± 0·09	13·06± 0·45
508–3	..	..	..	..	..	3	4	9	18	22	24	30	26	21	15	9	8	6	0	3	2	..	..	16·0— 31·8	22	22·85± 0·14	2·97± 0·10	13·27± 0·45
508–4	1	0	1	2	2	10	19	14	25	17	27	15	16	18	6	5	7	4	5	1	2	1	2	12·5— 34·0	22	22·23± 0·19	3·90± 0·13	17·54± 0·61

TABLE 10.—SUMMARY OF COMPARISONS BETWEEN THE MEAN WIDTHS AND LENGTHS OF SPORES OF FOUR MONOSPOROUS CULTURES OF *HELMINTHOSPORIUM SATIVUM* DERIVED FROM ONE MONOSPOROUS CULTURE

Cultures compared	Mean lengths		Mean widths	
	Difference in microns	Difference divided by the probable error of the difference	Difference in microns	Difference divided by the probable error of the difference
508–1 and 508–2	3·54±0·52	6·8	0·54±0·18	3·0
508–1 and 508–3	6·40±0·51	12·4	0·81±0·19	4·3
508–1 and 508–4	6·26±0·55	11·3	0·68±0·23	3·0
508–2 and 508–3	2·86±0·49	5·9	1·36±0·19	7·1
508–2 and 508–4	2·72±0·52	5·2	1·23±0·23	5·4
508–3 and 508–4	0·14±0·52	0·3	0·13±0·23	0·6

Four monosporous cultures from cultures 508-4 were made, and the cultural characteristics studied in the same manner. One of these second-generation monosporous cultures differed slightly in growth rate when compared with the other three cultures, which were identical in their cultural characteristics. Monosporous cultures were again made from one of these second-generation cultures, and the studies were continued. In this third generation the four monosporous cultures were identically the same (plate 3).

Unfortunately, it has not been possible to pursue the comparative morphological studies of these monosporous cultures further than the first generation, and at present the significance of these differences is not clear. Unless some error in sampling is still present in spite of the precautions taken to insure uniformity, the indications are that these monosporous cultures can be still further sub-

Comparative cultural characters of four monosporous cultures of *Helminthosporium* on three different media: Potato dextrose, agar (left), bean agar (centre), prune agar (right). Top to bottom—Cultures 1, 2, 3, 4.

divided into races differing from each other at least by slight but significant differences in morphology. This would suggest, although there is insufficient evidence for a definite conclusion, the frequent occurrence of mutations in *H. sativum*. Frequent occurrence of mutations in this fungus when grown on culture media has been shown recently by Christensen [9].

Comparative cultural studies were made with four cultures of *Fusarium* isolated from different wheat roots. The four cultures were very similar morphologically and were tentatively identified as *F. graminearum* Schwabe [*Gibberella Saubinetii* (Mont.) Sacc.]. Two of these cultures, I and III, had been found, in the pathogenicity studies described subsequently, to be very virulent pathogenes, while the other two cultures, II and IV, were much less virulent. The media used in these studies were potato dextrose agar, bean agar and boiled rice. The methods described in the cultural studies with *Helminthosporium* were followed closely. All of the plates were incubated at 22°C., and a comparison of the various cultures was made over a six-day period. From the cultural characteristics evidenced by the four cultures on the three media, each culture was distinctly different from any of the others. There were evidently four distinct physiological forms of *F. graminearum* present.

There was no evidence of any correlation between the cultural characteristics of these four forms of *Fusarium* and their virulence as pathogenes. In the case of the four forms of *Helminthosporium* previously described, there was no evident correlation between their pathogenicity and cultural characteristics.

Pathogenicity Studies with Fungi Isolated from Wheat Roots

The real significance of the results obtained from the culture work already described seemed to depend on the pathogenicity of the organism isolated. Experiments were undertaken to determine whether the fungi which had been found associated with wheat roots were saprophytic, weakly parasitic, or capable of causing severe injury under suitable conditions.

Relative pathogenicity of various fungi to wheat

Pathogenicity tests were carried out in the following manner: a well-mixed soil sample of the clay-loam type, sufficient to carry on the particular series in hand, was autoclaved for about two hours at fifteen pounds steam-pressure in 6-inch pots. The organism whose pathogenicity was being tested was grown in considerable quantity in quart jars on sterilized wheat. This inoculum was mixed liberally with the sterile soil and fifteen surface-sterilized seeds were planted in each pot. Three pots were inoculated with each organism. Marquis wheat was used throughout, the sample being hand-selected from a bulk sample. The seed was surface-sterilized by dipping in 50 per cent alcohol and then immersing in mercuric bichloride (1:1000 solution) for three minutes. The seed thus sterilized was washed in sterile water before planting. An average of 98 per cent of the seed handled in this manner germinated in blotting-paper tests. Nine to twelve organisms were compared in each series, and six series were carried through. The series were allowed to grow for about six weeks, during which time moisture conditions were kept approximately unchanged by watering repeatedly to maintain a constant weight. At the end of this time examination was made of the root systems. The soil was carefully washed from the roots and notes taken on the relative development of the plants and the browning of the roots. The data obtained from these tests are summarized in table 11. The relative top and root development in two series is shown in Plates 4 and 5.

Effect on the Top and Root Development of Marquis Wheat of Inoculating Soil with Organisms Isolated from Roots of Wheat.

(*Above*) Effect on top development. Series 5 (a). (Left to right)—*Fusarium, Fusarium, Verticillium, Fusarium, Fusarium, Fusarium, Check* (not inoculated).

(*Below*) Effect on root development. Representative root systems of three Marquis wheat plants grown in inoculated and uninoculated soil. Series 5 (a). (Left to right)—Arrangement same as in top figure.

Effect on the Top and Root Development of Marquis Wheat of Inoculating Soil with Organisms Isolated from Roots of Wheat.

(*Above*) Effect on top development. Series 5 (b). (Left to right)—Check (not inoculated), *Fusarium, Fusarium, Verticillium, Fusarium, Verticillium, Verticillium.*

(*Below*) Effect on root development. Representative root systems of three Marquis wheat plants grown in inoculated and uninoculated soil. Series 5 (b). (Left to right)—Arrangement same as in top figure.

TABLE II.—THE EFFECT ON MARQUIS WHEAT OF INOCULATING SOIL WITH VARIOUS FUNGI

Series	Organism	Number of seeds planted	Number of seedlings after 15 days	Condition of the plants at the end of the experiment		
				Top development	Bases of stems	Root development
1	Check	45	39	Healthy, vigorous	Clean	Extensive, no discoloration.
	Helminthosporium	45	39	Much reduced; stems weak and spindly.	Brown	Poorly developed, much discoloured.
	Fusarium	45	39	Good, vigorous and uniform.	Slightly or not at all discoloured.	Good, slightly discoloured.
	Fusarium	45	38	Poor, undeveloped	Slightly discoloured	Poor, very inadequate discoulored.
	Fusarium	45	38	Good, vigorous	Very slightly discoloured.	Medium, slightly discoloured.
2	Check	45	40	Good, healthy development.	Clean	Good, extensive, no discoloration.
	Botrytis[1]	45	42	Good, vigorous	Very slightly discoloured.	Good, slightly discoloured.
	Fusarium	45	15	Poor, much reduced	Several discoloured	Very poor, roots badly rotted, system seriously reduced.
	Helminthosporium	45	21	Very poor, very inadequate development.	Badly browned	Very poor, all but very young roots injured, entirely inadequate.
	Helminthosporium	45	39	Slightly underdeveloped	Slightly discoloured	Medium, considerably below normal, generally discoloured.
	Fusarium	45	37	Medium, not vigorous	Slightly discoloured	Good, slightly discoloured.
	Fusarium	45	41	Good development, uniform and vigorous.	Slightly discoloured	Medium, somewhat reduced, slightly discoloured.
	Fusarium	45	40	Poor, sickly appearance, underdeveloped.	Slightly discoloured	Poor, inadequate, considerable browning of roots.
	Fusarium	45	40	Medium, healthy appearance of stem and leaf.	Slightly browned	Medium, quite adequate in amount, slight discoloration.
	Fusarium	45	37	Plants vigorous	Slightly browned	Poor, meager development, much browned, quite inadequate.
	Fusarium	45	41	Medium	Slightly browned	Poor, development inadequate. Roots much browned.
	Fusarium	45	40	Good, healthy development of leaf and stem. Plants of good colour.	Clean	Good, very adequate in amount, some roots very slightly discoloured.
3	Check	45	41	Good colour, fairly vigorous growth.	Some stems slightly darkened.	Good, adequate in amount, slight discoloration on some roots.
	Fusarium	45	34	Medium, much the same as check.	Slightly discoloured	Poor, very much discoloured; quite inadequate in amount.
	Fusarium	45	41	Medium, plants strong but not uniform.	Occasionally discoloured	Medium, slightly discoloured.
	Fusarium	45	34	Much reduced, some plants badly injured.	Slightly discoloured	Poor, considerably discoloured.
	Fusarium	45	40	Uniform development, somewhat reduced.	Slightly discoloured	Poor, slightly discoloured, some roots badly browned.
	Fusarium	45	37	Medium	Considerably discoloured.	Poor, much discoloured and inadequate.
	Helminthosporium	45	19	Very poor, much reduced, weak and not uniform.	Blackened	Very poor, much browned, severe injury
	Fusarium	45	7	Very poor, sickly, not uniform.	Brown	Very poor, severely discoloured and much reduced.
	Fusarium	45	40	Good, vigorous and uniform.	Clean	Medium, some discoloration.
	Helminthosporium	45	24	Poor, very weak	Brown	Very poor, much discoloured.
4	Check	45	40	Good, uniform, vigorous	Clean	Good, roots abundant, no discoloration.
	Helminthosporium	45	32	Identical with Check	Clean	Good. Roots slightly discoloured.
	Helminthosporium	45	43	Good development	Clean	Good, slight discoloration.

TABLE II.—THE EFFECT ON MARQUIS WHEAT OF INOCULATING SOIL WITH VARIOUS FUNGI—
Concluded

Series	Organism	Number of seeds planted	Number of seedlings after 15 days	Condition of the plants at the end of the experiment		
				Top development	Bases of stems	Root development
4	Helminthosporium..	45	18	Variable; very much reduced to medium.	Slightly discoloured.....	Very variable. Roots in all cases discoloured.
	Helminthosporium..	45	36	Vigorous.....	Clean.....	Good, roots slightly discoloured and very abundant.
	Helminthosporium..	45	31	Vigorous.....	Stems occasionally discoloured.	Medium, somewhat discoloured and slightreduced.
	Helminthosporium..	45	26	Greatly reduced.....	Slightly discoloured.....	Generally reduced and discoloured.
	Helminthosporium..	45	22	Vigorous.....	Occasional stems slightly discoloured.	Medium, slightly discoloured, adequate in amount.
	Helminthosporium..	45	29	Very much reduced.....	Slightly discoloured.....	Poor, roots poorly developed and very badly discoloured.
	Helminthosporium..	45	37	Good, vigorous.....	Occasionally discoloured	Good, roots abundant, only occasionally discoloured.
	Helminthosporium..	45	34	Good, vigorous.....	Slightly discoloured.....	Medium, roots abundant, generally discoloured, and occasionally badly browned
5	Check.....	30	26	Good, normal vigorous growth.	Clean.....	Good, roots not discoloured, entirely adequate in amount.
	Fusarium.....	30	21	Good, vigorous.....	Clean.....	Good, very slightly discoloured.
	Fusarium.....	30	26	Vigorous.....	Occasionally slightly discoloured.	Medium, slightly discoured.
	Verticillium[1].....	30	27	Good.....	Clean.....	Very good, clean to slightly discoloured roots.
	Fusarium.....	30	12	Growth not uniform, vigorous.	Clean.....	Poor, slightly discoloured and underdeveloped.
	Fusarium.....	30	27	Medium, uniform.....	Clean.....	Slightly discoloured and under-developed.
	Verticillium[1].....	30	26	Good, vigorous.....	Clean.....	Good development, slight discoloration of roots.
	Verticillium[1].....	30	26	Good.....	Slightly discoloured.....	Medium, considerably discoloured and underdeveloped.
	Fusarium.....	30	20	Vigorous.....	Clean.....	Poor, much reduced.
	Verticillium[1].	30	28	Good, vigorous, uniform	Occasionally slightly discoloured.	Medium, slightly discoloured, considerably reduced.
	Fusarium.....	30	20	Medium, uniform growth.	Occasionally slightly discoloured.	Medium, inadequate, much discoloured.
	Fusarium.....	30	17	Reduced and not vigorous.	Slightly discoloured.....	Medium, discoloured, slightly underdeveloped.
	Fusarium.....	30	24	Vigorous growth.. ..	Slightly discoloured....	Medium, discoloured, much reduced.
6	Check.....	45	39	Good, vigorous.....	Clean.....	Good, adequate, not discoloured.
	Phoma[1].....	45	37	Identical with growth of check.	Clean.....	Good, adequate, not discoloured.
	Basidiomycete[1].....	45	17	Medium, considerably reduced.	Clean.....	Good, slightly discoloured, very adequate.
	Helminthosporium..	45	19	Medium, slightly reduced.	Slightly discoloured...	Adequate, severe browning.
	Alternaria.....	45	31	Good development.....	Clean.....	Good, adequate, not discoloured.
	Fusarium.....	45	34	Medium, uniform.....	Clean.....	Good, adequate, slightly discoloured.
	Verticillium[1].....	45	41	Medium, slightly reduced.	Clean.....	Good, no discoloration, roots abundant.
	Alternaria.....	45	30	Good, development slightly reduced.	Clean.....	Good, fairly adequate, slightly discoloured.
	Fusarium.....	45	32	Medium growth of stem and leaf.	Very slightly discoloured.	Good, slightly reduced. Some discoloration.

[1] Tentatively assigned to this group and certainly belonging to that general neighbourhood taxonomically.

Several interesting considerations are revealed by a study of table 11. In the first instance, it is interesting that the fungi which were really virulent parasites belonged to the genera *Fusarium* and *Helminthosporium*. All of the unknown or provisionally determined fungi were either weakly parasitic or non-pathogenic, under the conditions of the experiment. It is possible that these fungi may be more virulent under other conditions, but it seems more probable that they are to be considered for the most part saprophytic or weakly parasitic. Probably the incidence of their attack corresponds to the approaching maturity of the parts attacked.

There was an interesting difference in pathogenicity between the various cultures of *Fusarium* and *Helminthosporium* isolated. This was well brought out for different cultures of *Helminthosporium* in Series 4, where the pathogenicity of ten cultures was tested under approximately identical conditions. The cultures ranged from very virulent to weakly parasitic. The explanation is not immediately apparent, inasmuch as the cultures all appeared to be of the *sativum* type. The results, taken in conjunction with the cultural studies already considered, indicate the existence of many physiological strains of *H. sativum*, differing either in their pathogenicity or in their optimum temperature requirements.

A similar difference in pathogenicity among various cultures of *Fusarium* is evident in Series 3. The same explanation may apply here also, although in this case the matter of the specific identity of the cultures worked with has not been settled.

RELATIVE SUSCEPTIBILITY OF VARIOUS CEREALS TO THE SAME ORGANISM

It seemed desirable to determine whether the pathogenicity of the root-rotting organisms, particularly the *Fusaria* and *Helminthosporia*, was the same to other cereals as to wheat. For this purpose some preliminary tests were carried out.

A culture of *Helminthosporium* and one of *Fusarium*, which had been found to be pathogenic to wheat in previous experiments, were tested for virulence to wheat, oats, and barley. The procedure was the same as already outlined in the pathogenicity studies discussed in the previous section. The results are summarized in table 12. *Helminthosporium* was very pathogenic to wheat and barley, while *Fusarium* was markedly pathogenic only to wheat. The differences in growth of inoculated and uninoculated plants of oats were not sufficiently great to be of any significance. (See Plates 6 and 7.)

TABLE 12—RELATIVE SUSCEPTIBILITY OF WHEAT, OATS, AND BARLEY TO THE SAME CULTURES OF *HELMINTHOSPORIUM* AND *FUSARIUM*

Cereal	Soil inoculated with	Seeds planted	Plants alive after four weeks	Condition of the plants at the end of the experiment		
				Top development	Bases of stems	Root development
Marquis wheat	Helminthosporium	30	14	Medium, considerably reduced	Severely discoloured	Poor, entirely inadequate. Roots severely discoloured
	Fusarium	30	22	Good, vigorous	Many stems browned	Fairly adequate in amount, roots browned
	Check	30	30	Very good, vigorous	Clean	Excellent, very adequate. No discoloration
Victory oats	Helminthosporium	30	22	Medium, strong	Clean	Adequate, slightly browned. Injury not severe
	Fusarium	30	26	Good, fairly vigorous	Clean	Medium, not as adequate as check
	Check	30	29	Very vigorous	Clean	Adequate, occasional browning
Thorpe barley	Helminthosporium	30	11	Very poor	Severely discoloured	Inadequate, roots blackened, much reduced, severe injury
	Fusarium	30	23	Good, vigorous	Clean	As vigorous as check, slightly discoloured
	Check	30	27	Excellent, vigorous	Clean	Very adequate, occasional roots very slightly discoloured

Effect on the Top and Root Development of Wheat, Oats, and Barley of Inoculating Soil with *Fusarium*.

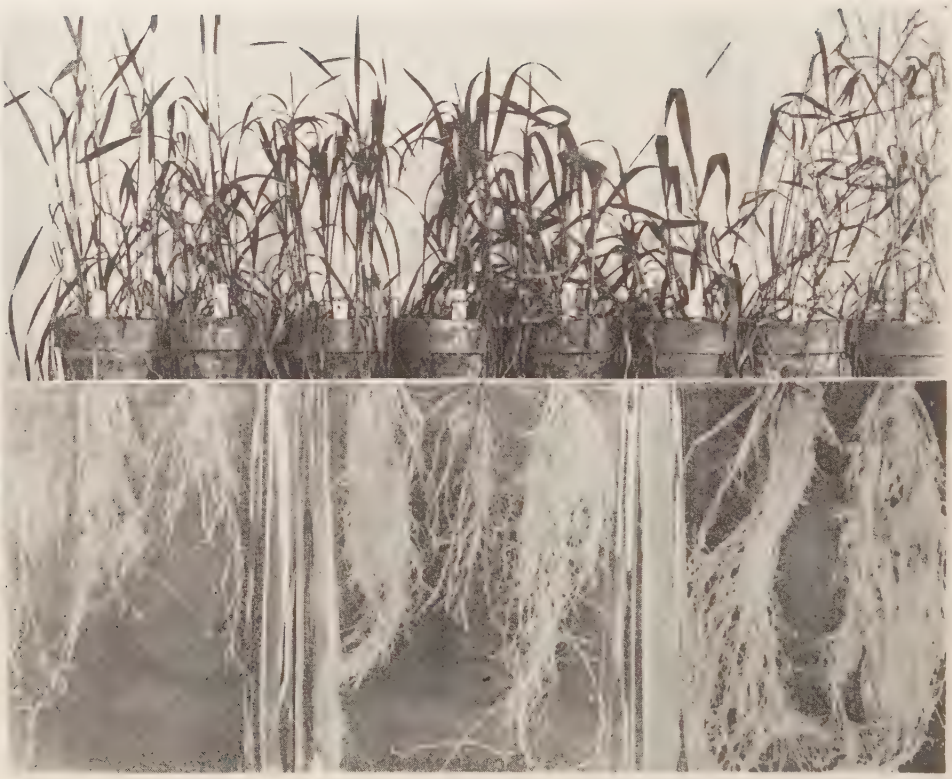
(*Above*) Effect on top development. (Left to right)—Wheat (inoculated), wheat (uninoculated), oats (inoculated), oats (uninoculated), barley (inoculated), barley (uninoculated).

(*Below*) Effect on root development. Representative root systems of three plants growing in inoculated and uninoculated soil. (Left to right)—Arrangement same as in top figure.

Effect on the Top and Root Development of Wheat, Oats, and Barley of Inoculating Soil with *Helminthosporium*.

(*Above*) Effect on top development. (Left to right)—Wheat (inoculated), wheat (uninoculated) oats (inoculated), oats (uninoculated), barley (inoculated), barley (uninoculated).

(*Below*) Effect on root development. Representative root systems of three plants growing in inoculated and uninoculated soil. (Left to right)—Arrangement same as in top figure.



Effect on the Top and Root Development of Marquis Wheat, Victory Oats, Thorpe Barley, and Rosen Rye, of Soil Inoculation with a Strain of *Fusarium* which was Virulent to Wheat.

(*Above*) Effect on top development. (Left to right)—Wheat (inoculated), wheat (uninoculated), oats (inoculated), oats (uninoculated), barley (inoculated), barley (uninoculated), rye (inoculated), rye (uninoculated).

(*Below*) Effect on root development. Representative root systems of three plants growing in inoculated and uninoculated soil. (Left to right)—Arrangement same as in top figure.

Effect on the Top and Root Development of Marquis Wheat, Victory Oats, Thorpe Barley, and Rosen Rye, of Soil Inoculation with a Strain of *Fusarium* which was Non-Virulent to Wheat.

(*Above*) Effect on top development. (Left to right)—Wheat (inoculated), wheat (uninoculated), oats (inoculated), oats (uninoculated), barley (inoculated), barley (uninoculated), rye (inoculated), rye (uninoculated).

(*Below*) Effect on root development. Representative root systems of three plants growing in inoculated and uninoculated soil. (Left to right)—Arrangement same as in top figure.

In a second experiment two cultures of *Fusarium*, one very pathogenic to wheat and one only weakly so, were tested for their relative pathogenicity to wheat, oats, barley and rye. The results are summarized in table 13. The culture which was so weakly pathogenic to wheat was likewise only weakly pathogenic to oats, barley, and rye. The culture which attacked wheat with much virulence was also fairly pathogenic to the other cereals. These results suggest that some of the *Fusaria* isolated may be at worst only weak parasites and of little significance in the root-rot problem (Plates 8 and 9). On the other hand, these apparently non-virulent cultures might prove to be very virulent under different environmental conditions.

TABLE 13—RELATIVE SUSCEPTIBILITY OF WHEAT, OATS, BARLEY, AND RYE TO TWO CULTURES OF *FUSARIUM* INOCULATED INTO SOIL

Cereal	Culture number	Seeds planted	Plants alive after eight weeks	Condition of the plants at the end of eight weeks		
				Top development	Bases of stems	Root development
Marquis wheat	Check	30	28	Vigorous, uniform development	Clean	No discolouration adequate in amount
	No. 6	30	15	Much reduced, stems spindly, leaves pale in colour.	Occasionally discoloured	Medium, many roots discoloured. Some roots dead
	No. 66	30	22	Vigorous, development	Clean	Good, adequate, slightly or not at all discoloured
Victory oats	Check	30	26	Good, strong	Clean	Good, not discoloured, very adequate
	No. 6	30	21	Medium, slightly reduced	Slightly discoloured	Some roots slightly infected, others badly discoloured and decidedly reduced.
	No. 66	30	25	Medium	Clean	Good, adequate in amount, not discoloured
Thorpe barley	Check	30	22	Medium development	Clean	Good, adequate, not discoloured
	No. 6	30	17	Somewhat reduced, not uniform	Considerably discoloured	Poor, reduced, much discoloured
	No. 66	30	24	Good, vigorous	Clean	Good, slightly or not at all discoloured, adequate
Rosen rye	Check	30	19	Good, uniform	Clean	Good, well developed and not discoloured
	No. 6	30	6	Poor development	Slightly discoloured	Poor, reduced, considerably discoloured
	No. 66	30	13	Medium, one per cent seriously injured	Clean	Good, reduced very slightly

CONTROL

Influence of Soil Sterilization on the Virulence of Attack

Soil was collected in the field from plots which had been fallowed, and had subsequently grown one, two, three, and five crops of wheat respectively. Four 6-inch pots of each soil type were collected. Two of these were autoclaved for four hours at 15 pounds steam-pressure, the other two were untreated. In each of the four pots, fifteen surface-sterilized seeds of Marquis wheat were planted. After six weeks' growth in the greenhouse, notes were taken on the number of plants, the average height, and the comparative root development in the various types of soil. These data are summarized in table 14.

TABLE 14—THE INFLUENCE OF STERILIZING FIELD SOIL WITH STEAM ON THE DEVELOPMENT OF WHEAT PLANTS

Soil: number of wheat crops grown since fallowing	Treatment	Seeds planted	Number of seedlings 10 days after planting	Condition of plants at the end of six weeks			
				Mean height (cms.)	Top development	Bases of stems	Root development
First crop.....	None.....	30	29	48·6	Slightly reduced, fairly vigorous	Slightly discoloured	Adequate in amount, slight browning of some roots
	Sterilized...	30	30	48·7	Healthy.....	Clean.....	Very adequate in amount. Very slight discoloration of some roots
Second crop....	None.....	30	26	48·45	Not as vigorous as in sterilized soil	Slightly discoloured	Slightly inadequate, considerable browning
	Sterilized...	30	28	50·2	Very vigorous and healthy	Very slight discoloration	Very healthy, adequate root system
Third crop.....	None.....	30	27	52·0	Not vigorous.....	All plants considerably discoloured	Inadequate, severe browning of all roots
	Sterilized...	30	29	55·2	Healthy and vigorous	Clean.....	No discoloration, very adequate
Fifth crop.....	None.....	30	26	47·0	Not vigorous, reduced	Severely discoloured	Not vigorous, inadequate in extent, much browning of roots
	Sterilized...	30	28	53·1	Vigorous and healthy	Clean.....	Adequate, no discoloration, healthy development

From a study of this table it is evident that in the untreated soil the effect so commonly met with in the field has not been reproduced. That is, there has been no progressive reduction in vigour of the resulting crop corresponding to the continued cultivation of wheat for several years on the same soil. In this experiment there was no reduction in vigour, even where the soil had produced five crops of wheat successively. But in the treated soil the influence of sterilization in the various cases is interesting. There was no apparent benefit in the case of the soil that had grown two crops of wheat since fallowing; but where three and five crops of wheat had been produced successively there was a very evident beneficial effect. How much of this can safely be attributed to the soil sterilization is rather problematic. Since sterilization of the soil of one and two crops following fallow did not cause any apparent increase in growth of the plants, it would seem that the increased vigour of the plants grown in soil of three and five years' successive wheat-cropping since fallow could not be attributed entirely to the removal of the parasitic organisms. Apparently some other factors are involved.

Relation of Seed Treatment to the Severity of Attack

Many workers in cereal pathology have recently reported evidence of increase in the germination of cereal seed following treatment with various chemicals [6, 16, 34]. Most of these chemicals have been the recently developed organic seed disinfectants which apparently consist very generally of complex salts of mercury. Semesan, one of these commercially-known compounds, was used in these preliminary tests to determine whether the stimulation would have any influence on the virulence of the attack of root-rotting organisms.

The test was made with a culture of *Helminthosporium* and one of *Fusarium*. Both of these were very pathogenic to wheat in previous experiments. Preparation of the soil and multiplication of inoculum were carried out as in the preced-

ing pathogenicity studies. Wheat, oats, and barley were used to test this treatment. Sufficient seed of each was treated with Semesan to plant four pots of inoculated, and two of uninoculated soil, which were used as checks. The treatment was as follows: the wheat was soaked for sixty minutes in a 0.25 per cent solution of Semesan; the oats for two hours in a 0.3 per cent solution; the barley for sixty minutes in a 0.3 per cent solution. The seed thus treated was allowed to dry thoroughly before planting. Concurrently, two pots of soil inoculated with *Helminthosporium*, two with *Fusarium*, and two uninoculated ones were planted with untreated seed.

The results are summarized in table 15. Treatment with Semesan stimulated the growth of young plants of oats, wheat, and barley. Barley was much more responsive to the treatment than wheat or oats. This was the case especially in the soil inoculated with *Helminthosporium*. The stimulation of plants from treated seed, even in the uninoculated soil, was very marked during the first three weeks of growth, but the effect was barely noticeable after the plants had grown for six weeks. Barley plants from treated seed in soil inoculated with *Helminthosporium* were much more vigorous than plants in the same soil type from untreated seed. Wheat plants were invigorated by seed treatment to a slight extent. Oats responded very little to the treatment. In the case of wheat and barley, Semesan appears to have some value as a seeding stimulant, as it permits the growth of a more vigorous root system which can better withstand the attack of the organisms.

Although some positive results were obtained from this experiment, the general technique employed, and the methods of soil inoculation are, after all, quite artificial. Many factors, such as the amount of inoculum introduced into the soil and the addition of excessive quantities of foreign material to the soil make it difficult to obtain results which can be interpreted and compared with conditions as they occur in nature. Greenhouse soil-inoculation studies indicate the possible value of extensive field studies with seed treatments for the partial control of root-rot diseases. The control of these important diseases depends on more than one method. Treating seed with the recently developed mercury seed-disinfectants is only one of the methods open for investigation in an attempt to control these diseases. The value of seed treatment has still to be determined under field conditions.

DISCUSSION AND CONCLUSIONS

It is evident that root-rotting fungi are very commonly present in all the major wheat-producing areas of Manitoba. These fungi can be expected to cause serious damage locally, whenever environmental conditions favour their attack. Judging from plant-disease survey reports and from the increasing attention given to the root-rot problem, such suitable conditions are occurring with greater frequency. The investigations to date do not offer any specific means of control, and they seem to indicate that little relief can be hoped for from ordinary crop rotation, which is sometimes recommended as a control measure. Clean cultural practices to prevent the accumulation of infected material in the soil, and the use of a systematic crop rotation should greatly assist in checking the ravages of the foot- and root-rot organisms. It seems probable that the abundance of organic matter present in Manitoba soils will permit these fungi to persist indefinitely as saprophytes. This possibility lends additional complications to securing effective control measures.

The factors influencing the severity of attack of these fungi are very imperfectly understood. Definite physiologic races of varying virulence are involved. Environmental conditions exert a profound influence on the pathogenicity of these definite forms. Christensen [9] has recently distinguished in culture at least thirty-seven physiologic forms of *Helminthosporium sativum*. Not

TABLE 15—THE EFFECT ON THE CONTROL OF HELMINTHOSPORIUM AND FUSARIUM OF TREATING SEED OF WHEAT, OATS AND BARLEY WITH SEMESAN

Cereal	Soil inoculated with	Seed treatment	Number of seeds planted	After 20 days		Condition of plants at the end of the experiment (65 days)				
				Seedlings developed	Mean height of seedlings cms.	Number still living	Mean height of plants cms.	Top development	Bases of stems	Root development
Marquis wheat	Helminthosporium	Treated	30	17	17·0	17	37·1	Reduced, not uniform	Discoloured, some stems blackened.	Much reduced, inadequate, roots severely blackened.
		Untreated	30	15	15·5	14	34·2	Growth very weak	Badly discoloured	Very greatly reduced, entirely inadequate.
	Fusarium	Treated	30	29	17·5	22	38·6	Vigorous growth	Slightly discoloured	Roots badly discoloured, slightly inadequate.
		Untreated	30	26	16·5	21	38·1	Uniform, vigorous development.	Few stems severely browned	Slightly discoloured, normal development.
	Check	Treated	30	15	17·9	15	47·0	Very vigorous	Clean	Very adequate, vigorous development.
		Untreated	30	14	16·8	12	45·7	Medium development	Slight browning of stems	Considerable discoloration.
Victory oats	Helminthosporium	Treated	30	30	19·7	29	41·2	Normal, vigorous growth.	Some stems blackened	Severely discoloured, slightly reduced.
		Untreated	30	29	16·2	28	38·0	Medium, considerably reduced.	Very light browning of stems	Severely browned, much reduced.
	Fusarium	Treated	30	30	19·7	29	34·2	Normal vigorous growth	Slightly discoloured	Adequate, slight discoloration.
		Untreated	30	30	15·4	28	35·9	Vigorous, not uniform	Some stems badly discoloured.	Good root development, slight browning.
	Check	Treated	30	29	20·9	28	39·4	Normal vigorous growth	Clean	Very adequate, no discoloration.
		Untreated	30	30	18·9	29	39·3	Medium, bad colour	Clean	Slight discoloration, slightly reduced.
Thorpe barley	Helminthosporium	Treated	30	28	16·4	25	34·3	Medium, slightly reduced development.	Few plants blackened at bases of stems.	Reduced, severely discoloured.
		Untreated	30	25	7·8	11	27·3	Very poor, severe injury, 14 plants killed.	Severely blackened, injury severe.	Greatly reduced, entirely inadequate, severely discoloured.
	Fusarium	Treated	30	25	21·5	25	39·7	Medium, few plants dwarfed	Two plants severely blackened.	Good, very adequate, slight discoloration.
		Untreated	30	30	21·8	25	39·6	Not uniform, considerably reduced.	Many stems browned	Slightly reduced, generally discoloured.
	Check	Treated	30	27	18·3	27	36·1	Very healthy and vigorous, uniform.	Clean	Very good, adequate, very slight discoloration.
		Untreated	30	27	17·8	26	34·6	Normal development	Slight browning of some stems.	Development good, slight discoloration.

only did these physiologic forms differ in general appearance on culture media, but they were very different physiologically. The most important point, however, was that they differed pathogenically. He also found asexual mutations occuring frequently from some of these forms. The mutants differed from their parents not only in morphological characters but also in pathogenicity. Such conditions increase the complexity of an already complicated problem.

The sharply delimited areas in which virulent attacks of foot- and root-rots commonly occur suggest that the conditions governing their attack are definite and precise. It is evident from this preliminary investigation that the problem lies largely with those organisms belonging to the genera *Helminthosporium* and *Fusarium*. Regardless of what crop is grown in a rotation, these fungi appear to be universally present. There is also an accumulation of fungi other than *Fusarium* and *Helminthosporium* in the wheat soils of Manitoba. Fortunately, most of these fungi are either non-pathogenic or very weakly so. Their occurrence might readily be a significant factor in the reduction of plant vigour and in the marked decrease in yields. The remarkably high percentage of wheat plants infected with foot- and root-rotting fungi is exceedingly significant. It suggests that one of the most serious phases of the problem may be the ever-present non-virulent type of infection which reduces the root system and hence the vitality of the plant, giving little if any marked symptoms above ground. With from sixty to ninety per cent of the wheat plants from widely scattered areas infected in this manner, the significance of this phase of the root-rot problem is worthy of consideration.

The magnitude and pressing significance of the problem in Manitoba is established by these preliminary investigations. Manitoba, in common with the other parts of the spring-wheat region, must recognize the foot- and root-rot problem as a limiting factor in wheat production, and must make a determined effort to cope with it. Investigations along every phase of the problem should be continued. Particular attention should be given to selecting resistant varieties and to studying the relation of soil factors to the incidence of attack by foot- and root-rotting organisms.

SUMMARY

1. The objects of the investigations were chiefly to determine: (*a*) the fungous flora of wheat roots in Manitoba; (*b*) the influence of various crop rotations on the fungous flora of wheat roots and (*c*) the pathogenicity of the fungi from wheat roots.

2. A large number of pure-culture isolations from wheat roots collected in various parts of the province showed that *Fusarium* and *Helminthosporium* were very widely and consistently associated with wheat root-rots in Manitoba.

3. A preliminary survey in 1925 indicated that true take-all, *Ophiobolus cariceti* (Berkely and Broome) Saccardo, was fairly common in certain parts of the province. Further survey work is necessary to determine just how widespread and destructive it is.

4. The results of two years' work with ten different rotation series did not show any conspicuous correlation between the severity of foot-rot infection and the cultural practice involved. There does not seem to be any marked tendency for root-rotting organisms to accumulate in soil during six years' continuous wheat-cropping.

5. The specific identity of the *Fusaria* isolated from wheat roots has not been determined. The only species of *Helminthosporium* isolated was *H. sativum*.

There were evidently several physiologic forms of this organism, distinct from each other either in morphology of spores, culture characteristics, or pathogenicity.

6. Twenty-eight cultures of *Fusaria* were tested for their pathogenicity in the greenhouse. The differences in the pathogenicity of these cultures were very great. Some were very virulent, while others were relatively non-virulent.

7. The pathogenicity of sixteen cultures of *H. sativum* was tested. There were remarkable differences in the virulence of these cultures. Pathogenicity studies of ten cultures of other root-infesting fungi, belonging to five widely different genera, demonstrated that they were non-pathogenic or only slightly pathogenic under the conditions of the experiment.

8. The various isolations of *Fusarium* and *Helminthosporium* were found to vary a great deal in their pathogenicity. Some cultures were very virulent parasites. None of the fungi isolated from wheat roots, except those belonging to the *Fusarium* and *Helminthosporium* groups, were at all virulent under the experimental conditions.

9. Seeds of wheat, oats, and barley, treated with Semesan and planted in inoculated soil, produced more vigorous seedlings than did the untreated seed. The stimulation was most pronounced in barley. In soil inoculated with *Helminthosporium* a higher percentage of barley seedlings from treated seed arrived at maturity than from untreated seed, and the mature plants were more vigorous.

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